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In utero exposure to triphenyltin disrupts rat fetal testis development

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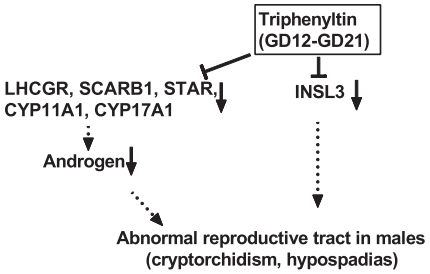
HIGHLIGHTS

- In utero exposure to triphenyltin inhibits androgen production of male fetus.
- Triphenyltin increases fetal Leydig cell aggregation.
- Triphenyltin downregulates steroidogenesis related gene expression.

GRAPHICAL ABSTRACT



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ABSTRACT

Triphenyltin is an organotin that is widely used as an anti-fouling agent and may have endocrine-disrupting effects. The objective of the current study was to investigate effects of triphenyltin on the development of rat fetal testis. Female pregnant Sprague Dawley dams were gavaged daily with triphenyltin (0, 0.5, 1, and 2 mg/kg body weight/day) from gestational day 12 to day 21. Triphenyltin dose-dependently decreased serum testosterone levels (0.971 ± 0.072 and 0.972 ± 0.231 ng/ml at 1 and 2 mg/kg, respectively) from control level (2.099 ± 0.351 ng/ml). Triphenyltin at 1 and 2 mg/kg doses also induced fetal Leydig cell aggregation, decreased fetal Leydig cell size and cytoplasmic size. Triphenyltin decreased the expression levels of *Lhcgr*, *Scarb1*, *Star*, *Cyp11a1*, *Cyp17a1*, *Insl3*, *Fshr*, *Pdgfa*, and *Sox9* by 0.5 mg/kg dose and above. However, triphenyltin did not affect Leydig and Sertoli cell numbers. In conclusion, the current study indicated that in utero exposure of triphenyltin disrupted fetal Leydig and Sertoli cell development.

1. Introduction

Testicular dysgenesis syndrome (TDS) refers to a wide manifestation spectrum of reproductive disorders in males with fetal origin, including cryptorchidism (undescended testis) and hypospadias (urethral abnormalities) in newborn baby boys and testicular cancer and infertility or subfertility in adult men (Skakkebaek

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et al., 2001). The cause of TDS is not completely clear, but the environmental factors may play an important role (Hu et al., 2009). TDS might happen because of fetal Leydig and Sertoli cell developmental failure due to the exposures to endocrine disruptors. In the prenatal testis, the testicular interstitium is populated by fetal Leydig cells, which produce two important hormones, androgen and insulin-like peptide 3 (INSL3, encoded by *Insl3* gene)[see review (Wen et al., 2016)]. In the rat testis, fetal Leydig cells emerge in the interstitial compartment from gestational day (GD) 12 and begin to secrete androgens on GD 14, and produce androgen robustly on GD 19 (Traore et al., 2016). Androgen production in the fetal testis starts with cholesterol, which is transported into fetal Leydig cells from high density lipoprotein via scavenger receptor class B member 1 (SCARB1, encoded by *Scarb1* gene). Intracellular cholesterol is required to be transported into the inner membrane of the mitochondrion by steroidogenic acute regulatory protein (STAR, encoded by *Star* gene) for the catalysis by a cytochrome P450 enzyme, cholesterol side-chain cleavage enzyme (CYP11A1, encoded by *Cyp11a1* gene). CYP11A1 converts cholesterol via a series of enzymatic reactions in the mitochondrion into steroid pregnenolone, which diffuses out into neighboring smooth endoplasmic reticulum, where two important steroidogenic enzymes are located: 3β-hydroxysteroid dehydrogenase 1 (HSD3B1, encoded by *Hsd3b1* gene) and cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17A1, encoded by *Cyp17a1* gene). HSD3B1 converts pregnenolone into progesterone and CYP17A1 converts progesterone into androstenedione (Wen et al., 2016). Interestingly, another important steroidogenic enzyme, 17β-hydroxysteroid dehydrogenase 3 (HSD17B3, encoded by *Hsd17b3* gene) is located in fetal Sertoli cells in rodents (Ye et al., 2017), which catalyzes androstenedione into testosterone. Androgen, produced by a combination of fetal Leydig and Sertoli cells, is essential for the development of the male reproductive tract and urinary tract.

In addition to androgen, INSL3 is another important factor produced by fetal Leydig cells that is essential for the descent of testis. INSL3 binds to the leucine-rich repeat-containing G protein-coupled receptor 8 in the gubernaculum to facilitate the initial phase of fetal testis descent into their extrabdominal position (Adham et al., 2000b; Anand-Ivell et al., 2009). Androgen plays an important role in the late stage of testis descent. In the fetal testis, the development of fetal Leydig cells does not require luteinizing hormone (LH), but these cells do express LH receptor (LHCGR, encoded by *Lhcgr* gene) and are capable of responding to LH stimulation. Fetal Leydig cell development is also regulated by fetal Sertoli cell-secreted factors. In the male fetus, the Y chromosome-linked mammalian sex-determining factor (SRY) upregulates SOX9 in fetal Sertoli cells to trigger fetal testis differentiation (Sekido and Lovell-Badge, 2008). The fetal Sertoli cells express follicle-stimulating hormone (FSH) receptor (FSHR, encoded by *Fshr* gene), which is essential for the cells to respond to the pituitary-secreted FSH. Fetal Sertoli cells secrete several important factors to regulate the development of fetal reproductive tract, including anti-müllerian hormone (AMH, encoded by *Amh* gene) that not only regulates the regression of müllerian tube but also regulates fetal Leydig cell development (Behringer, 1995; Josso et al., 2012) and Desert Hedgehog (DHH, encoded by *Dhh* gene) that regulates fetal Leydig cell differentiation (Yao et al., 2002). Therefore, disruption of the developmental processes of either fetal Leydig or Sertoli cells could be a major cause behind TDS, including cryptorchidism and/or other alterations in the trajectory for testicular development and function in the postnatal period.

Many endocrine disruptors could interfere with fetal Leydig and Sertoli cell development. One of these is triphenyltin (TPT). TPT contains a tin atom that is bound covalently to three phenyl groups (Graceli et al., 2013). TPT is widely used in various industrial products, such as anti-fouling agent for ships and boats, fungicides, and plastic stabilizers (Shawky and Emons, 1998). Due to its widespread use as a preservative in marine coatings, TPT is a common pollutant in marine and freshwater ecosystems. TPT toxicity may be biomagnified via food chain upon its poisoning to barnacles, algae, and other organisms. Studies indicate that TPT may be an endocrine disruptor for the reproductive system since it was found that exposure to the reagent resulted in imposex in many aquatic gastropods, in which females have parts of the male reproductive system, including the penis and vas deferens superimposed on a normal female genital system. However, its implications for the development of mammalian reproductive tract are less explored. One study indicated that TPT exposure to rats via gavage from GD 0 to GD 3 or from GD 4 to GD 6 produced implantation loss (Ema et al., 1997). Although there is no report for its effect on the development of fetal Leydig cells, study indicated that TPT had toxic effects on the male reproductive system in the adult animals (Reddy et al., 2006). TPT significantly decreased sperm count in the epididymal tract, sperm motility, and sperm viability, as well as lowered serum testosterone levels, which is associated with decreases in HSD3B1 and HSD17B3 activities. Since TPT significantly increased serum FSH and LH levels, its effects on the male reproductive system were primary at testicular level (Reddy et al., 2006). *In vitro* short-term TPT treatment also down-regulated pig *Cyp17a1* expression without affecting *Star*, *Cyp11a1*, *Hsd3b1*, and *Hsd17b3* mRNA levels (Nakajima et al., 2005). However, none of these studies have focused on the perinatal period, a sensitive period for secondary sex differentiation in the rats for TPT toxicity. In the current study, we investigated effects of gestational exposure to TPT on the development of fetal testis (including fetal Leydig and Sertoli cells) in the rat model with a focus on the sensitive masculinization window.

2. Materials and methods

2.1. Animals

Adult female and male Sprague Dawley rats were purchased from Shanghai Laboratory Animal Center (Shanghai, China). Rats were allowed one week to acclimate prior to mating. Female and male pairs were randomly selected and mated. After pregnancy was confirmed, female rats was housed individually in an IVC cage at 23 ± 2 °C, with relative humidity of 55 ± 5%, in a 12-hr light-dark cycle environment. The experimental protocol for animal toxicity study was approved by Wenzhou Medical University Laboratory Animal Ethics Committee and in conformity with procedures described in the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Animal treatment

TPT chloride was purchased from Sigma (St. Louis, MO). TPT was suspended in corn oil (vehicle control). Each pregnant rat was gavaged daily with 0.5 ml TPT at 0 (control, corn oil), 0.5, 1, or 2 mg/kg body weight from GD 12 to GD21 (n = 6 rats/group). The doses were selected based on a previous study (WIL-Research-Laboratories, 1985) that found TPT, at a dose range of 0.35–8 mg/kg, was safe to the general health of the pregnant rats. The body weights of pregnant rats were recorded daily. Dams were euthanized by CO_2 at GD 21 and body weights of dams and male pups were measured. Blood of male each pup was collected and serum was gathered after centrifugation. Sera from male pups of the same dam were pooled together. One set of rat testes (at least one testis per male fetus per pregnant rat) was randomly selected and frozen